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E-Article

The Adaptive Value of Stress-Induced Phenotypes: Effects of Maternally Derived Corticosterone on Sex-Biased Investment, Cost of Reproduction, and Maternal Fitness

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abstract: The question of why maternal stress influences offspring phenotype is of significant interest to evolutionary physiologists. Although embryonic exposure to maternally derived glucocorticoids (i.e., corticosterone) generally reduces offspring quality, effects may adaptively match maternal quality with offspring demand. We present results from an interannual field experiment in European starlings (*Sturnus vulgaris*) designed explicitly to examine the fitness consequences of exposing offspring to maternally derived stress hormones. We combined a manipulation of yolk corticosterone (yolk injections) with a manipulation of maternal chick-rearing ability (feather clipping of mothers) to quantify the adaptive value of corticosterone-induced offspring phenotypes in relation to maternal quality. We then examined how corticosterone-induced "matching" within this current reproductive attempt affected future fecundity and maternal survival. First, our results provide support that low-quality mothers transferring elevated corticosterone to eggs invest in daughters as predicted by sex allocation theory. Second, corticosterone-mediated sex-biased investment resulted in rapid male-biased mortality resulting in brood reduction, which provided a better match between maternal quality and brood demand. Third, corticosterone-mediated matching reduced investment in current reproduction for low-quality mothers, resulting in fitness gains through increased survival and future fecundity. Results indicate that the transfer of stress hormones to eggs by low-quality mothers can be adaptive since corticosterone-mediated sex-biased investment matches the quality of a mother to offspring demand, ultimately increasing maternal fitness. Our results also indicate that the branding of the proximate effects of maternal glucocorticoids on offspring as negative ignores the possibility that short-term phenotypic changes may actually increase maternal fitness.

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Although the proximate effects of maternally derived steroid hormones on offspring phenotype have garnered significant attention from ecological physiologists for the last 15 years (e.g., Schwabl 1993; Groothuis et al. 2005*a*, 2005*b*), virtually nothing is known about how these maternal effects influence fitness (Groothuis et al. 2005*b*; Love et al. 2005, 2008). Moreover, almost all of these studies have focused on the effects of yolk androgens (reviewed in Groothuis et al. 2005*b*), despite there being numerous steroids that are excellent candidates for causing numerous life-history trade-offs (Love et al. 2005). A particularly good example are glucocorticoids, such as corticosterone in birds, reptiles, and amphibians, which mediate adaptive physiological and behavioral responses to "stressful" events (Wingfield et al. 1998; Sapolsky et al. 2000; Wingfield 2005) within the larger context of maintaining daily homeostatic energetic balance (Harvey et al. 1984; Dallman et al. 1993; Remage-Healey and Romero 2001). As such, corticosterone can be linked to an individual's energetic state (Holberton et al. 1996; Love et al. 2005, 2008; Kitaysky et al. 2006; Blas et al. 2007; Love and Williams 2008). Importantly, embryos in a wide range of taxa are sensitive to maternally derived glucocorticoids during development and express many phenotypic adjustments (fish: McCormick 1998, 1999; reptiles: Sinervo and DeNardo 1996; Meylan and Clobert 2005; Lovern and Adams 2008; birds: Hayward and Wingfield 2004; Love et al. 2005; Saino et al. 2005; Hayward et al. 2006; Love and Williams 2008; mammals: Seckl 2001, 2004). Again, however, the result of many of these studies has been the branding of effects as simply positive or negative to the offspring, largely ignoring the possibility that proximate adjustments in offspring may provide fitness costs/benefits to mothers in both the present and the future (Love et al. 2005; Love and Williams 2008). Moreover, while many of the proximate effects of maternally derived glucocorticoids have

been documented in laboratory systems (reviewed in Seckl 2001, 2004; Seckl and Meaney 2004; Gluckman et al. 2005; Macrì and Würbel 2006), this field has not attempted to quantify the adaptive value of glucocorticoid-induced phenotypes (Dufty et al. 2002). However, recent studies in oviparous vertebrates have begun to interpret the phenotypic effects of maternally derived glucocorticoids on offspring as potentially adaptive mechanisms linking the mother's environment to that of the developing young (de Fraipont et al. 2000; Love et al. 2005, 2008; Meylan and Clobert 2005; Love and Williams 2008; Love et al. 2008). Unlike the mammalian fetus, which has continuous feedback with the mother via the placenta, the maternal transfer of hormones to eggs in species like birds, egg-laying reptiles, and fish represents a "sealed bid" scenario where mothers have only one opportunity between laying and hatching to transfer information about the quality of the future environment. As such, oviparous species provide a tractable system for understanding the evolution of maternally derived hormonal effects, since the effects of maternal reproductive decisions and offspring plasticity on offspring phenotype can theoretically be partitioned.

Love et al. (2005, 2008) recently suggested that maternally derived corticosterone could act as an adaptive mechanistic link between maternal quality, sex-biased maternal investment in offspring, and maternal fitness. The goal of the current study was to use a manipulative approach to test this hypothesis in a free-living, facultatively polygynous cavity-nesting bird, the European starling (*Sturnus vulgaris*). Males grow more quickly during postnatal development (Love et al. 2005; Verspoor et al. 2007) and are larger as adults (Cabe 1993; Verspoor et al. 2007), with size being important in competition for nest sites (Flux and Flux 1992). Sons are therefore predicted to have higher reproductive variance than females and are theoretically evolutionarily more costly for low-quality mothers to produce than daughters (i.e., Trivers and Willard 1973; Love et al. 2005). We began by creating specific nestling phenotypes via a manipulation of yolk corticosterone in eggs, a treatment designed to mimic the amount of hormone transferred to eggs by poor-condition mothers (Love et al. 2005, 2008). The direct yolk manipulation avoids any potential behavioral side effects associated with elevating the hormone in the mother. We then examined the success of the corticosterone-induced offspring phenotypes under postnatal environments of differing qualities (by reducing or maintaining maternal chick-provisioning capability using primary feather clipping; Rowland et al. 2007) to quantify the adaptive value of these offspring phenotypes (Pfennig and Murphy 2000; Dufty et al. 2002). Our goals were to examine the effects of maternally derived corticosterone on sex allocation and then to ask whether hormone-induced allocation within the current brood increases maternal fitness via changes in future fecundity and maternal survival. This study therefore addresses four distinct questions. (1) Do the phenotypic effects of maternally derived corticosterone act as a mechanism for sex allocation in low-quality mothers (sensu Trivers and Willard 1973; Cameron and Linklater 2002)? To answer this question, we measured sex-specific effects of the treatments on offspring phenotype across multiple traits: behavioral effects (begging), developmental effects (body mass, growth, and structural size), effects on physiological systems (cell-mediated immune responses), and changes in hatching sex ratio. (2) Do corticosterone-induced phenotypic changes in offspring proximately match offspring demand to maternal rearing capacity, thereby reducing the investment in current reproduction? To answer this question, we examined whether corticosterone-induced changes to offspring reduced maternal provisioning rates and increased maternal condition. (3) Does a corticosterone-induced matching of offspring to mother increase investment in future maternal condition and fecundity, as predicted by life-history trade-off theories (sensu Stearns 1992)? As such, we followed mothers raising second, unmanipulated broods (no hormone manipulation) in the same season to document within-season effects of the original manipulation on maternal condition and reproductive output and to measure future fecundity in the following year. (4) Does exposing offspring to maternal corticosterone by low-quality mothers in the current reproductive stage increase the probability of a mother surviving to reproduce again? To answer this question, we examined long-term consequences of the original manipulations on local maternal survival following our experimental manipulation. Because the data presented in this article are part of a larger multifaceted study examining the effects of developmental stress on multiple systems, some of the results naturally overlap with those presented in another recently published article (Love and Williams 2008). We therefore refer to that article where necessary to avoid unnecessary overlap.

Methods

Field Site and Hormonal Manipulation of Yolk Corticosterone

The focal study was conducted at the Davistead Dairy Farm in Langley, British Columbia, Canada (49°10′N, 122°50′W) from April to July of 2005, and mothers were then followed through to 2006 and 2007 to measure maternal survival and future fecundity. Our colony consists of 254 nest boxes that are mounted on posts around pastures and on farm buildings used by a wild population of European starlings (mean ± SE; clutch size: 5.9 ± 0.2

eggs; incubation length: 10.3 ± 0.1 days; postnatal period: 21 ± 0.6 days; Love et al. 2008). Nest boxes were checked daily to determine clutch initiation, laying sequence, and clutch completion dates. On the morning of the laying of the first egg for a given female, individuals were randomly (within the colony) assigned to either an oil injection (hereafter referred to as "sham"; $n = 34$ females) or corticosterone injection ($n = 34$ females) treatment group (treatments paired each day to control for laying date) within the synchronous 7–8-day peak of laying of first clutches in our colony. Within 3 h of a new egg being laid, it was removed from the nest, the injection site was cleaned with ethyl alcohol, and the injection was made into the yolk. The hole was sealed using cyanoacrylate glue (Loctite Superglue Control Gel, Henkel), the egg's laying order was marked, and the egg was measured and returned to the nest. Corticosterone-treated eggs were injected with 10 μL of a 1,277 ng/mL corticosterone solution (Sigma C2505) dissolved in sterilized sesame oil (Sigma S3547) so that the manipulation elevated mean yolk corticosterone concentrations by 1.5 SD from the population mean (from 15.4 to 28.3 ng/g, as reported in Love et al. 2008; average fresh yolk weight of 1.012 ± 0.008 g; $n = 163$ eggs). Likewise, sham-injected eggs were handled similarly but were injected with only the sesame oil vehicle.

Manipulation and Measurement of Maternal Chick-Rearing Ability

On day 8 of incubation, individual females were caught and again split pairwise within each yolk hormone treatment (controlling for laying date) into either a feather-clipping treatment ($n = 32$ females) designed to reduce maternal provisioning rates (Winkler and Allen 1995; Hill 2003; Rowland et al. 2007) or an unmanipulated treatment (captured and handled only: $n = 32$ females). The feather-clipping treatment consisted of removing the ninth, sixth, and third primary feathers; the sixth and third secondary feathers; and the sixth and third rectrices (tail feathers) at the base of each feather. Control birds were merely captured, measured, and released. All females were measured (beak length, tarsus length, wing cord, and body mass), banded with metal and color bands, and released to return to normal activities. Mothers were recaught at day 7–8 of chick rearing to determine posttreatment changes in body mass in relation to the treatments. By combining the hormonal and maternal treatments, we produced four overall treatments: (1) corticosterone-exposed nestlings raised by feather-clipped mothers (herein referred to as CORT-clipped, or B_C), (2) corticosterone-exposed nestlings raised by nonclipped mothers (CORT-nonclipped, or B_{NC}), (3) sham-exposed nestlings raised by feather-clipped mothers (sham-clipped, or S_C), and (4) sham-exposed nestlings raised by nonclipped mothers (sham-nonclipped, or S_{NC}). To assess parental provisioning rates (nestling-rearing ability), we performed a 30-min behavioral observation of each nest box per day over 2 consecutive days using spotting scopes when nestlings were 6–8 days of age (during the linear phase of postnatal growth). Provisioning rates are reported as the number of feeding visits per chick per hour for each parent based on the mean brood size of the nest for the 2-day observation period (following Chin et al. 2005; Rowland et al. 2007).

Phenotypic Responses and Survival of Nestlings

At day 10 of incubation (within 0.5 days of hatching), clutches were removed and placed in an incubator for approximately 6–10 h until hatching; artificial eggs were used to maintain maternal incubation behavior. Immediately following hatching (before being returned to their nests), all nestlings underwent a begging test protocol to assess (1) total begging time and (2) maximum begging intensity to quantify the effects of the hormone treatment on begging performance. Nestlings were induced to beg using a simultaneously applied mechanical (tap near the nest cup) and auditory (whistle) cue designed to replicate the arrival of the mother at the nest box. Total time spent begging was recorded using a stopwatch, while maximum begging intensity was scored from 1 (lowest) to 5 (highest) following the protocol outlined by Leonard et al. (2000); each chick was tested three times. All nestlings were then weighed and measured (exposed culmen, metatarsus, wing), and a small blood sample was collected for polymerase chain reaction (sex) analysis. Measurements were repeated at the ages of 5, 10, 15, and 17 days; we began measuring flattened wing cord at 10 days of age when primary feathers started to appear and again at 15 and 17 days of age. Nestling identity and subsequent age were tracked using nontoxic food coloring and chick-specific feather clipping until 10 days of age, at which time all chicks were banded with metal bands (permit 10646) so that individual nestlings could be identified.

All nestlings underwent a phytohemagglutinin (PHA) test at 17–18 days of age as a means of evaluating the cell-mediated immunity (CMI) of individuals (Martin et al. 2006) and as previously used in this population (Chin et al. 2005; Love et al. 2005; Rowland et al. 2007). We injected 50 μg of PHA (PHA-p; Sigma: L-9132) in 50 μL of sterile phosphate buffered saline subcutaneously with a 27-gauge needle into the right wing web (patagium) of each bird. Patagium thickness was measured three times to 0.01 mm before and again 24 h after injection using a gauge micrometer (Dyer, model 304-196). Cell-mediated immunity to PHA was calculated as the change in thickness of the wing web before and following injections, as outlined by

Smits et al. (1999). Repeatability of both initial ($r = 0.91$, $P < .0001$) and final ($r = 0.87$, $P < .0001$) measurements was high, and we used mean values of the three measurements.

Molecular Sexing

The blood sample at hatching was collected on a piece of filter paper and stored frozen at −20°C. On the basis of techniques reported by Love et al. (2005), nestling sex was determined using polymerase chain reaction amplification. DNA was isolated from the blood samples using InstaGene matrix (Bio-Rad Laboratories, cat. no. 732–6030) and from tissue samples collected from unhatched eggs using DNeasy kits (Qiagen) following manufacturer's protocols. Polymerase chain reaction amplification was carried out in a total volume of 10 μL and run using the P2 (5′-TCTGCATCGCTAAATCCTTT) and CW (5′-AGAAATC-ATTCCAGAAGTTCA) primers followed by digestion with HaeIII enzyme.

Statistical Analysis

Since the goal of this study was to determine whether exposing offspring to maternally derived corticosterone was adaptive for a low-quality mother, we pay particular attention in the "Results" and "Discussion" to differences between the CORT-clipped and sham-clipped groups. The remaining control groups (CORT-nonclipped and sham-nonclipped) allow us to (1) examine the fitness of a low-quality mother if her quality can increase from laying to chick rearing (CORT-nonclipped) and (2) compare all of the manipulated treatments with an unmanipulated baseline control mother that raises normal young (sham-nonclipped). To examine hatching traits, we statistically analyzed data in relation to the hormone treatment only, since hatchlings had not yet been exposed to the effects of their mother's treatment.

We used ANOVA to analyze potential differences between females assigned to the various treatments (body mass, clutch size) as well as differences in maternal/paternal provisioning rates. We used repeated-measures ANOVA to examine changes in maternal body mass and reproductive parameters between reproductive attempts (repeated statistic is referred to as "time" in "Results"). We used general linear mixed models (GLMMs) to analyze sex-specific effects of the hormonal and overall treatments on nestling traits (body mass/size and CMI) by including nestling sex and the relevant treatment as fixed factors; maternal identity was included as a random factor to control for nonindependence due to the inclusion of siblings in the analysis, and both laying sequence and clutch size were included as covariates since clutch-specific laying sequence patterns of yolk corticosterone have been found (Love et al. 2008). Post hoc comparisons for significant treatment effects were carried out using the sequential Bonferroni post hoc procedure, with the P value corrected for the number of pairwise comparisons made depending on the type of analysis (Rice 1989); for the sex × treatment interaction, post hoc comparisons were made within sexes. Sex ratio of the brood (a measure of sex-specific mortality), as a function of maternal treatment, was analyzed using GLMM with a binomial error structure; maternal identity was included as a random factor, and laying sequence was included as a covariate (Love et al. 2005). Sex-specific mortality as a function of maternal treatment and in relation to laying sequence was analyzed within sexes using a GLMM with laying sequence included as a covariate. For all GLMM models involving sex ratio/mortality, the significance of the explanatory variables was determined by their Wald statistic using the χ^2 distribution with α set to 0.05 (Crawley 1992).

Results

First, all mothers were randomly assigned to the treatments, that is, no differences in laying date, egg mass, clutch size, structural size, body mass, and wing loading and no differences in return rates following the clipping procedure (for details, see Love and Williams 2008). Second, maternal and parental provisioning rates were significantly lower in the sham-clipped group compared with all other groups (Love and Williams 2008; fig. 1*A*). Finally, feather-clipped mothers lost more body mass than non-feather-clipped mothers, regardless of hormonal treatment (Love and Williams 2008; fig. 1*B*).

Effects of Hormonal Treatment on Offspring Phenotype

Corticosterone treatment affected embryonic development in sons but not daughters (Love and Williams 2008). Sons exposed to corticosterone were significantly lighter (but not structurally smaller) at hatching than sham-exposed sons, while daughters were unaffected, controlling for egg mass (sham males: 5.26 ± 0.06 g; CORT males: 5.03 ± 0.03 g; sham females: 5.14 ± 0.04 g; CORT females: 5.10 ± 0.04 g; for details, see Love and Williams 2008). Corticosterone-exposed offspring begged with higher intensities than sham-exposed offspring (hormone treatment: $\chi^2_1 = 4.88$, $P = .027$), and sons exposed to corticosterone begged for less total time (sex × hormone treatment: $\chi^2_1 = 6.36$, $P = .012$). Both begging time and intensity showed high repeatability across the three trials (time: $r = 0.65$, $P < .001$; intensity: $r = 0.72$, $P < .001$). Daughters of sham-clipped mothers were lighter and structurally smaller at fledging than daughters in all other

groups; sons showed no effects of either treatment combination on fledging body mass, although sons raised by clipped mothers were structurally smaller (Love and Williams 2008; table 1). Cell-mediated immunity varied in a complex, sex-specific manner across treatments (sex × treatment: $\chi^2_3 = 8.48$, $P = .03$; table 1); sham-nonclipped daughters had lower responses than daughters from all other treatment groups, and sons from the CORT-clipped and sham-nonclipped groups had lower responses than sham-clipped and CORT-nonclipped sons. No laying order effects were found for any of the hatching or fledgling traits discussed above (all $P > .29$).

Treatment Effects on Current Reproductive Effort

We found no effect of the corticosterone or clipping treatment on hatching success ($F = 1.24$, df $= 1, 66$, $P = .26$; sham: 86.1% ± 6.6%, corticosterone: 76.18% ± 5.8%), hatching brood sizes, or hatching brood sex ratios (fig. 2; see Love and Williams 2008) in first broods in 2005. However, brood sizes and sex ratios changed significantly during postnatal development in a treatment-specific manner (fig. 2). Nestlings raised by clipped mothers had higher mortality than nestlings raised by nonclipped mothers, and mortality was male biased in the CORT-clipped group (fig. 2*B*; see Love and Williams 2008). This resulted in a significant sex-biased decrease in fledging brood size for the CORT-clipped group (fig. 2*A*; see Love and Williams 2008). Furthermore, survival from hatching to fledging was dependent on the laying order of the eggs from which they hatched in sons raised by clipped mothers (treatment × laying order: $\chi^2_5 = 13.5$, $P = .01$; fig. 2*C*) but not in daughters ($P > .56$). We could not determine whether this was due to the synergistic effects of the corticosterone treatment together with the higher levels of the hormone that are naturally present in later-laid eggs (see Love et al. 2008) or whether this is an effect of hatching order per se, since laying and hatching order were correlated in this study ($r = 0.36$, $P < .01$).

Effects of the Experimental Manipulation on Fecundity in Second Broods

The treatment a mother experienced during her first brood had significant residual effects on both her and her offspring's quality during second broods in 2005 (figs. 1, 2; table 2). We found no treatment differences for maternal return rates from first to second broods (treatment: $\chi^2_3 = 1.46$, $P = .68$), laying interval between broods (treatment: $\chi^2_3 = 2.26$, $P = .52$), egg size (time × treatment: $F = 0.075$, df $= 3, 24$, $P = .97$), or clutch size (time × treatment: $F = 1.17$, df $= 3, 26$, $P = .34$) for second clutches, although second clutches of all females

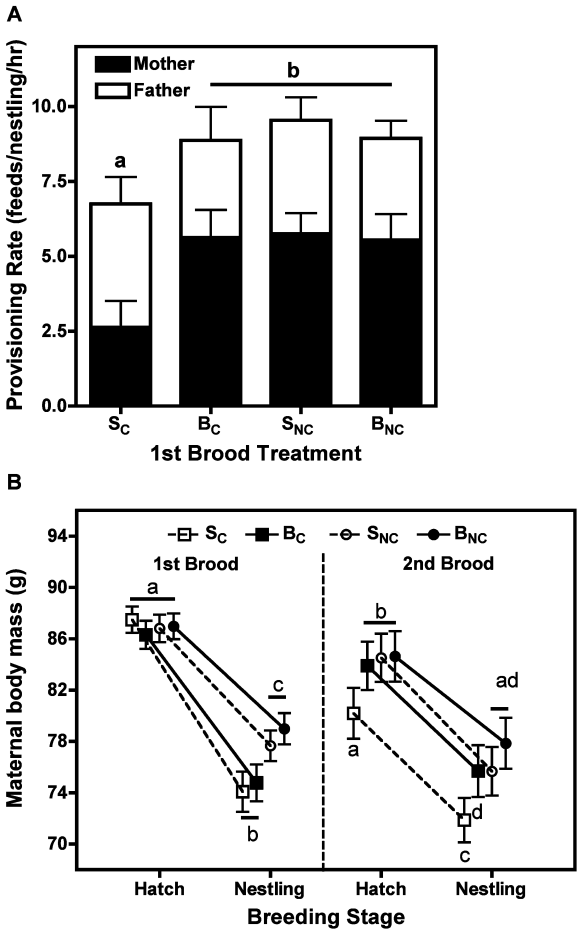

Figure 1: Treatment effects on (*A*) maternal and parental provisioning rates in first broods and (*B*) maternal body mass changes during chick rearing in first and second broods of European starling mothers (mean ± SEM; different letters represent significant differences between groups; treatments: sham-clipped [S_C], CORT-clipped [B_C], sham-nonclipped [S_{NC}], and CORT-nonclipped [B_{NC}]).

were smaller than first clutches (time: $F = 36.81$, df $= 1, 26$, $P < .0001$). However, sham-clipped mothers initiated and ended second clutches with significantly lower body masses (time × treatment: $F = 3.26$, df $= 3, 19$, $P < .05$; post hoc: all $P < .025$; fig. 1*B*).

The postnatal phenotype of nestlings in second broods was also influenced by the treatment combination their mothers experienced during their first breeding attempt (table 2). Although hatching success in second broods was similar across all treatments ($\chi^2_3 = 0.37$, $P = .94$), hatching mass was significantly higher in nestling of sham-clipped mothers compared with all the other treatments (treatment: $\chi^2_3 = 11.3$, $P = .01$; post hoc: all $P < .018$), and sons in general were larger than daughters ($\chi^2_1 = 3.7$, $P = .05$; post hoc: all $P < .025$). However, despite

Table 1: Effects of maternal and hormonal treatments on fledgling phenotypic traits in male and female starling nestlings in first broods in 2005

Traits	S_C	B_C	S_{NC}	B_{NC}
Males:				
Body mass (g)	$76.42 \pm .91^A$	77.53 ± 1.12^A	$74.37 \pm .98^A$	$76.64 \pm .91^A$
Tarsus (mm)	$34.40 \pm .30^A$	$34.48 \pm .37^A$	$35.16 \pm .32^B$	$35.26 \pm .30^B$
Wing cord (mm)	$91.47 \pm .88^A$	91.48 ± 1.09^A	$93.45 \pm .96^B$	$93.63 \pm .89^B$
CMI (mm × 10)	19.59 ± 1.52^A	15.97 ± 1.87^B	14.00 ± 1.65^B	19.90 ± 1.52^A
Females:				
Body mass (g)	70.86 ± 1.00^A	75.07 ± 1.02^B	$74.03 \pm .90^B$	$75.38 \pm .94^B$
Tarsus (mm)	$33.85 \pm .33^A$	$34.67 \pm .34^B$	$34.86 \pm .30^B$	$34.46 \pm .31^B$
Wing cord (mm)	$89.06 \pm .97^A$	92.00 ± 1.00^B	$92.32 \pm .88^B$	$91.49 \pm .92^B$
CMI (mm × 10)	20.76 ± 1.67^A	20.03 ± 1.72^A	15.58 ± 1.51^B	20.67 ± 1.58^A

Note: Different letters represent significant differences within sexes across treatments. CMI, cell-mediated immunity. Treatments: sham-clipped (S_C), CORT-clipped (B_C), sham-nonclipped (S_{NC}), and CORT-nonclipped (B_{NC}).

hatching with heavier body masses, sons raised by sham-clipped mothers were lighter and structurally smaller at fledging than sons raised by mothers from all other treatments, controlling for initial hatching mass (body mass: time × sex: $\chi^2_3 = 14.4$, $P = .0024$; post hoc: all $P < .02$; tarsus: time × sex × treatment: $F = 2.52$, df $= 12, 53$, $P = .01$; post hoc: all $P < .01$; wing cord: sex × treatment: $F = 2.34$, df $= 6, 44$, $P = .05$; post hoc: all $P < .025$; table 2); daughters were unaffected in body mass and tarsus, although those raised by CORT-clipped mothers had shorter wing cords (post hoc: all $P < .025$; table 2). Furthermore, CMI was highest in nestlings raised by CORT-clipped mothers, controlling for fledging body mass, regardless of sex (treatment: $\chi^2_3 = 68.2$, $P < .0001$; post hoc: all $P < .009$; table 2), and overall, sons fledged with lower CMI than daughters (sex: $\chi^2_1 = 15.1$, $P < .0001$; post hoc: all $P < .01$; table 2).

First-brood maternal treatment of mothers also affected the breeding success of these mothers in second broods. Fewer sham-clipped mothers fledged at least one nestling from second broods (treatment: $\chi^2_3 = 9.16$, $P = .027$; post hoc: all $P < .017$; fig. 3*A*). Second, when mothers were able to raise at least one fledgling, sham-clipped mothers fledged significantly fewer offspring compared with mothers in the other treatments (despite having similar hatching brood sizes; treatment: $\chi^2_3 = 8.33$, $P = .04$; post hoc: all $P < .019$; fig. 3*B*).

Long-Term Effects of Treatment on Fecundity, Survival, and Maternal Fitness

The experimental treatment experienced by mothers during their first broods in 2005 had significant residual effects on local maternal survival and future fecundity the following 2 years and, hence, maternal fitness (we were able to measure fecundity in only 2006 broods; figs. 4–6; table 3). Mothers assigned to the sham-clipped treatment in 2005 had the lowest local survival of all of the original 2005 treatments (2006: $\chi^2_3 = 8.89$, $P = .03$; post hoc: all $P < .025$; 2007: $\chi^2_3 = 8.31$, $P = .05$; post hoc: all $P < .03$; fig. 4). Furthermore, original sham-clipped mothers that survived to 2006 laid significantly smaller eggs than did mothers from all other treatments ($\chi^2_3 = 20.5$, $P = .0001$; post hoc: all $P < .001$; fig. 5*A*), although clutch sizes were similar (time × treatment: $F = 1.75$, df $= 3, 14$, $P = .21$; fig. 5*B*). Fledgling traits were differentially affected by the previous maternal treatment: while nestling body mass did not differ between treatments (treatment: $\chi^2_3 = 1.66$, $P = .64$; table 3), nestlings of original sham-clipped mothers were structurally smaller than nestlings raised by mothers from all other original treatments (tarsus: $\chi^2_3 = 11.7$, $P = .008$; post hoc: all $P < .01$; wing cord: $\chi^2_3 = 9.66$, $P = .02$; post hoc: all $P < .021$; table 3). Finally, fledging success was lowest in sham-clipped mothers (treatment: $\chi^2_3 = 7.99$, $P = .05$), leading to significantly lower reproductive output, as measured by the number of successful fledglings produced per mother (treatment: $\chi^2_3 = 8.54$, $P = .036$; post hoc: all $P < .018$; fig. 5*B*). We calculated the cumulative average number of independent offspring produced by a mother exposed to each treatment by combining the average number of offspring produced by mothers in each treatment over the three recorded breeding attempts (2005 first and second broods, 2006 first broods) with the return and success rates in 2005 second broods and 2006 first broods. Using this "cumulative average number of independent offspring produced" as a proxy for maternal fitness indicates that sham-clipped mothers had lower fitness than CORT-clipped mothers through their third reproductive attempt postmanipulation (treatment × time interaction: $\chi^2_2 = 6.12$, $P < .05$; post hoc: all $P < .03$; fig. 6).

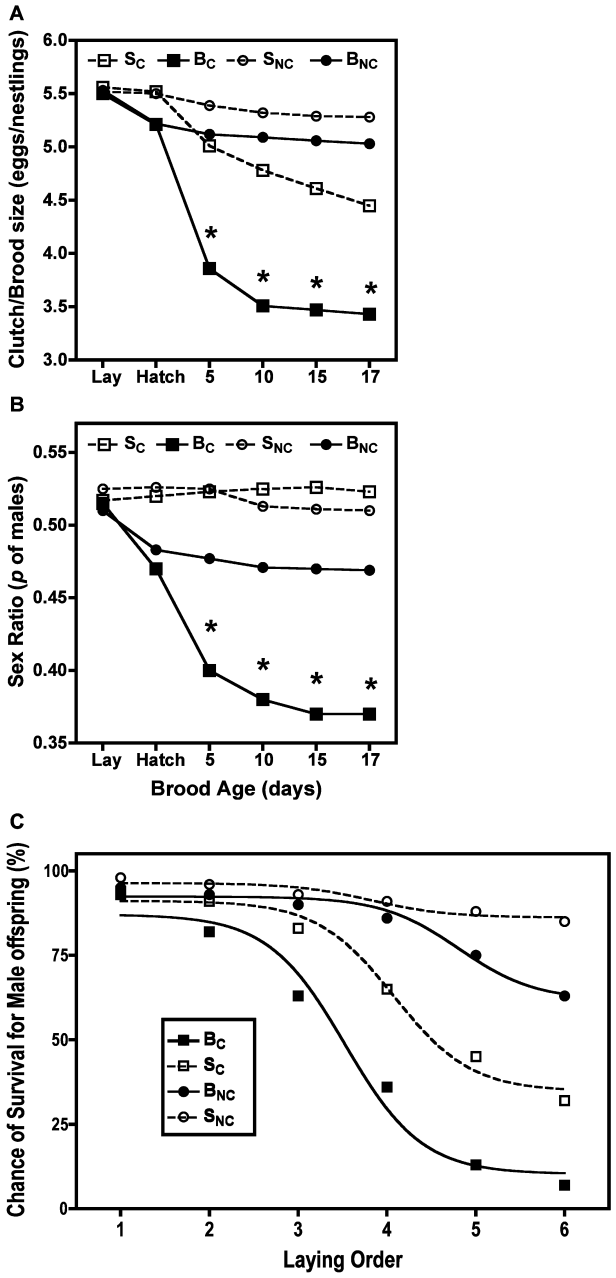

Figure 2: Treatment effects on (*A*) changes in brood size, (*B*) sex ratio (proportion of males), and (*C*) male survival in relation to laying order during postnatal development in first broods of European starling mothers (treatments: sham-clipped [S_C], CORT-clipped [B_C], sham-nonclipped [S_{NC}], and CORT-nonclipped [B_{NC}]; asterisks represent significant differences between B_C and all other treatment groups).

Discussion

Sham-clipped mothers showed reduced maternal provisioning ability and were therefore "mismatched" to the relative demand of their unmanipulated brood, leading to a reduction in daughter body mass and structural size at fledging. Exposure to elevated prenatal corticosterone reduced the hatching mass and begging of sons, and CORT-clipped mothers experienced rapid, male-biased brood reduction. We argue that these smaller broods provided feather-clipped mothers with a better "match" between their offspring-rearing capability and their brood's demand. As a result, these mothers produced both high-quality sons and daughters at fledging. This assertion is supported by data indicating that the "mismatched" mothers began and ended second broods with lower body masses than "matched" mothers. As a result, raising corticosterone-exposed offspring in effect "rescued" feather-clipped mothers during the current breeding attempt and resulted in an increase in future fecundity and survival prospects compared with mismatched mothers. Increased fecundity and survival resulted in higher fitness (calculated as the average cumulative number of independent offspring produced for a mother) for matched mothers compared with mismatched mothers. We examine evidence supporting this idea that low-quality mothers that expose offspring to elevated corticosterone are investing in evolutionarily less expensive daughters, as predicted by sex allocation theory (Trivers and Willard 1973). We then further explore evidence that the sex-specific phenotypic changes in offspring exposed to elevated yolk corticosterone lead to brood reduction, which proximately reduces current reproductive effort for low-quality mothers, ultimately increasing maternal fitness through both increased survival and future fecundity. Finally, we discuss whether it is (1) selection for plasticity in embryonic growth in males of sexually size-dimorphic species or (2) maternal control over embryonic developmental pathways in sons that is responsible for the phenotypic responses of sons to elevated yolk corticosterone.

Influence of Yolk Corticosterone on Sex-Specific Allocation

Trivers and Willard (1973) were the first to formally propose the idea that maternal quality should influence the level of investment in offspring when the fitness returns differ for the two sexes. Assumptions of sex allocation theory include predictions for both sex-biased investment and sex ratio adjustments for low-quality mothers: mothers in poorer condition would be favored if they produced more of the less costly sex and invested more in this sex (Cameron and Linklater 2002). Since glucocorticoids are tightly coupled to energetic state through their prominent role in homeostatic energy balance (see the first section of this article), maternal corticosterone acting through phenotypic effects on offspring has been proposed to provide an adaptive link between maternal and offspring qualities (Love et al. 2005, 2008; Meylan and Clobert 2005).

Table 2: Effects of first brood maternal treatment on fledgling phenotypic traits in male and female starling nestlings in second broods in 2005

Traits	S_C	B_C	S_{NC}	B_{NC}
Males:				
Body mass (g)	66.55 ± 3.23^A	73.53 ± 2.70^B	75.49 ± 1.94^B	73.17 ± 2.38^B
Tarsus (mm)	$34.16 \pm .47^A$	$35.11 \pm .63^B$	$35.16 \pm .28^B$	$35.24 \pm .35^B$
Wing cord (mm)	84.33 ± 2.52^A	88.45 ± 2.28^B	90.10 ± 1.69^B	89.94 ± 2.09^B
CMI (mm × 10)	13.23 ± 2.68^A	36.42 ± 5.08^B	14.62 ± 1.89^A	12.02 ± 1.52^A
Females:				
Body mass (g)	72.09 ± 3.09^A	70.48 ± 2.68^A	67.53 ± 1.79^A	70.39 ± 2.59^A
Tarsus (mm)	$34.58 \pm .45^A$	$33.95 \pm .52^A$	$34.65 \pm .26^A$	$34.16 \pm .38^A$
Wing cord (mm)	88.61 ± 2.50^A	82.09 ± 3.06^B	90.52 ± 1.56^A	90.09 ± 2.26^A
CMI (mm × 10)	14.91 ± 2.61^A	41.29 ± 3.10^B	21.04 ± 1.68^A	17.57 ± 2.28^A

Note: Different letters represent significant differences within sexes across treatments. CMI, cell-mediated immunity. Treatments: sham-clipped (S_C), CORT-clipped (B_C), sham-nonclipped (S_{NC}), and CORT-nonclipped (B_{NC}).

In our previous work (Love et al. 2005), starling mothers implanted with corticosterone before laying deposited the hormone into yolks, resulting in an investment in less expensive daughters, whereby sons showed increased embryonic mortality, reduced hatching masses, and reduced postnatal growth. These results suggested that the deposition of yolk corticosterone would benefit mothers in poor condition by providing an adaptive mechanistic link between maternal quality and sex-specific allocation. Deposition of maternally derived corticosterone to eggs in relation to maternal condition would be a beneficial bet-hedging strategy in stochastic environments where the correlation between environmental cues at laying (and therefore potentially maternal condition) and conditions during chick rearing might be low and unpredictable (Love et al. 2005). The direct yolk corticosterone manipulation used in this study specifically tests whether it is yolk corticosterone per se that is responsible for the documented changes in sex allocation rather than potential behavioral/physiological side effects associated with elevating the hormone in the mother. Results of this study are supported by recent work in captive avian species indicating that males show reduced hatching masses and slower postnatal growth in response to exposure to elevated yolk corticosterone (Hayward et al. 2006; Satterlee et al. 2007). We would predict that if low maternal quality at laying continues into chick rearing, then lighter-hatched sons should experience increased mortality, since low mass at hatching has a significant negative effect on survival during early postnatal development in altricial birds when postnatal conditions are harsh (reviewed in Williams 1994); the outcome would be a further relative investment in daughters via a reduction in competition by sons. In support of these predictions, we found that brood sex ratios of CORT-clipped mothers were significantly female biased by fledging (0.36 male/female ratio) compared with sham-clipped mothers (0.52 male/female ratio), indicating an indirect investment in daughters in the hormone-exposed group. Moreover, CORT-clipped mothers directly invested in daughters through increases in daughter quality (likely mediated via a decrease in the number of competing brothers), where these daughters were significantly heavier and structurally larger than daughters of sham-clipped mothers and the least physiologically stressed (lower baseline plasma corticosterone; Love and Williams 2008) of all groups. We also predicted that if maternal quality improved following laying, mothers adopting the flexible strategy of exposing young to yolk corticosterone could fledge both good-quality sons and daughters, given that small males are expected to catch up during postnatal development (Love et al. 2005). This prediction was also confirmed in this study: offspring of CORT-nonclipped mothers fledged with the same quality as did offspring of sham-nonclipped mothers, despite corticosterone-exposed sons hatching at smaller body masses compared with their sham counterparts. Moreover, offspring of CORT-nonclipped mothers did not experience significant mortality compared with offspring of CORT-clipped mothers and in fact showed similar survival to offspring of sham-nonclipped mothers.

Few studies have examined either short- or long-term sex-specific effects of maternal glucocorticoids on offspring phenotype in free-living vertebrates. Sinervo and DeNardo (1996) reported that an elevation of maternal corticosterone in side-blotched lizards (*Uta stansburiana*) resulted in larger female offspring at hatch compared with sham-implanted females with no effect on male offspring. Meylan and Clobert (2005) reported sex-specific effects of maternal corticosterone treatment in the common lizard (*Lacerta vivipara*; although hormone elevations in this study were likely pharmacological rather than physiological), with male offspring showing apparent negative

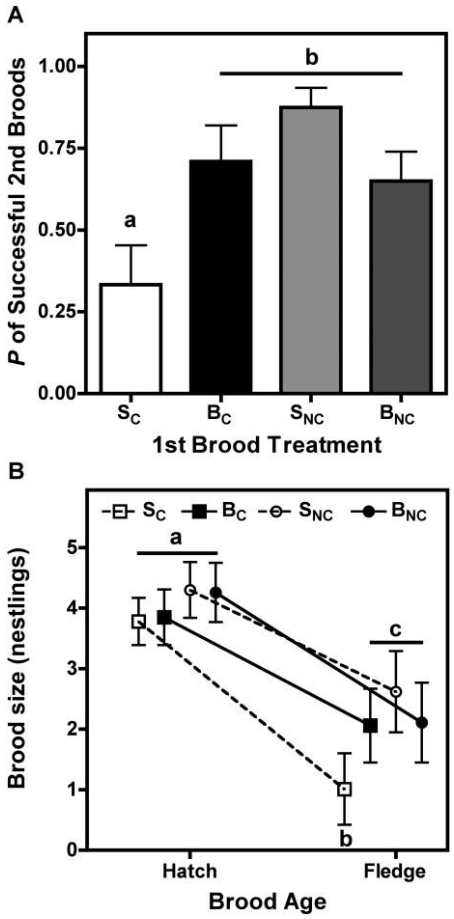

Figure 3: Treatment effects on (*A*) the proportion of successful second broods (at least one chick surviving to fledging age) and (*B*) brood size during postnatal development in second broods of European starling mothers (mean ± SEM; different letters represent significant differences between groups; treatments: sham-clipped [S_C], CORT-clipped [B_C], sham-nonclipped [S_{NC}], and CORT-nonclipped [B_{NC}]).

growth effects of the hormonal treatment while at the same time exhibiting positive effects on their survival. Hayward et al. (2006) reported that male Japanese quail (*Coturnix coturnix japonica*) were more developmentally sensitive (slower postnatal growth) than females following exposure to experimentally elevated yolk corticosterone. Finally, Satterlee et al. (2007) recently reported that prenatal corticosterone exposure negatively affected reproductive function in male Japanese quail. In this study, male offspring exposed to corticosterone hatched at lower body masses and begged at lower rates at hatching than did sham-exposed males, traits that likely impacted survival probability. However, sons of CORT-clipped mothers that survived to fledging showed no impacts on fledging body mass, structural size, or baseline plasma corticosterone levels (Love and Williams 2008). Nonetheless, we found that sons of CORT-clipped mothers had lower CMI compared with sham-clipped counterparts, suggesting an obligate cost to the male immune system following exposure to maternal/environmental stress (Chin et al. 2005; Love et al. 2005; Rubolini et al. 2005). However, this effect was not present in sons of CORT-nonclipped mothers, strongly indicating that costs of corticosterone exposure on the immune system are indirect and context dependent rather than a direct result of corticosterone-induced suppression of the immune system. Instead, it may be that CORT-exposed sons must "catch up" during development (Metcalfe and Monaghan 2001), resulting in a redirection of resources away from the immune system (Love et al. 2005). To make things even more complex, we found that even offspring of sham-clipped mothers had higher CMI than did sham-nonclipped offspring, further suggesting that passerine nestlings may have some capacity to increase CMI in response to stressful rearing conditions. Regardless, together with our previous work (Love et al. 2005), results from this study indicate that maternal corticosterone can act as an hormonal mechanism adaptively modifying sex allocation decisions in relation to maternal quality.

Carryover Effects of Yolk Corticosterone on Maternal Fitness

It has been proposed that maternal corticosterone modulates the cost of reproduction (Sinervo and DeNardo 1996; Love et al. 2005), given that glucocorticoids play a central role in the regulation of allostatic load during reproduction (Wilson and Wingfield 1992; Sinervo and

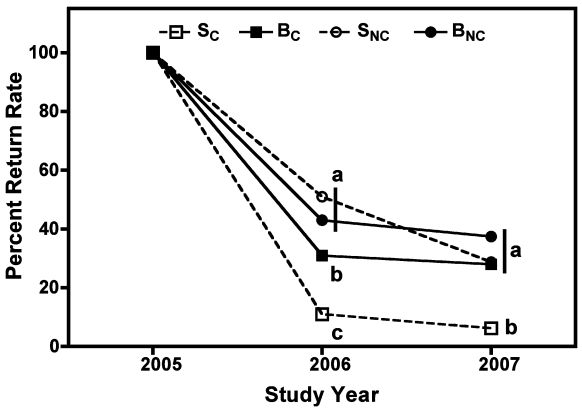

Figure 4: Effects of the 2005 treatment on local survival of European starling mothers to 2006 and 2007 (different letters represent significant differences between groups within years; treatments: sham-clipped [S_C], CORT-clipped [B_C], sham-nonclipped [S_{NC}], and CORT-nonclipped [B_{NC}]).

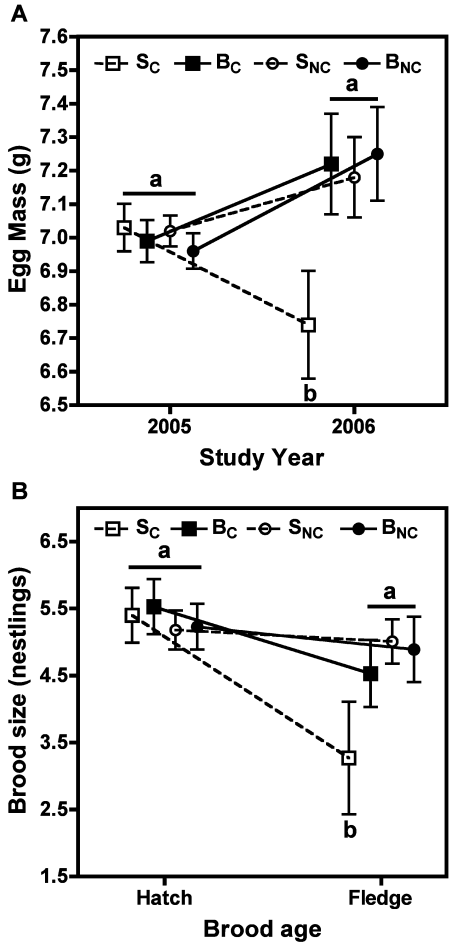

Figure 5: Effects of the 2005 treatment on (*A*) egg mass in 2006 and (*B*) brood size changes in 2006 of European starling mothers (mean ± SEM; different letters represent significant differences between groups; treatments: sham-clipped [S_C], CORT-clipped [B_C], sham-nonclipped [S_{NC}], and CORT-nonclipped [B_{NC}]).

DeNardo 1996; Love et al. 2004; Boonstra 2005; Wingfield 2005). The cost of reproduction is a central concept in evolutionary biology, where increased investment in current reproduction is predicted to lead to a decrease in maternal condition, future fecundity, and even survival (Williams 1966; Stearns 1992; Williams 2005). According to theories on costs of reproduction (Williams 1966; Reznick 1985) and data from empirical studies (Nur 1984, 1988; Orell and Koivula 1988; Dijkstra et al. 1990; Daan et al. 1996; Young 1996; Golet et al. 1998, 2004), the corticosterone-mediated decrease in brood size for CORT-clipped (matched) mothers should represent a reduction in maternal energetic investment in the current reproductive attempt compared with sham-clipped (mismatched) mothers. This energetic saving of raising a smaller brood benefits CORT-clipped mothers in three ways: (1) increased body condition at both the beginning and the end of the second (within-year) reproductive attempt, (2) increased future fecundity (e.g., more young, better-quality young) in both second (within-year) and future (across-year) broods, and (3) increased survival to future breeding attempts. First, we found that CORT-clipped mothers lost less body mass raising nestlings in their first (focal) broods compared with sham-clipped mothers. Moreover, CORT-clipped mothers began and ended their second broods in better condition than did sham-clipped mothers. This supports the hypothesis that maternally derived corticosterone hormonally mediates the trade-off between reproductive investment and maternal condition (Stearns 1992). Second, twice as many CORT-clipped mothers successfully fledged at least one young from second broods compared with sham-clipped mothers (CORT-clipped: 67.8%, sham-clipped: 31.2%), and when nests were successful, CORT-clipped mothers fledged more young than did sham-clipped mothers (despite mothers in both treatments having laid similar-sized second clutches). Furthermore, second-brood sons of original CORT-clipped mothers also fledged in better condition (significantly heavier, structurally larger, and higher CMI responses) than did sons from sham-clipped mothers; daughters of CORT-clipped mothers had longer wing cords and higher CMI. Perhaps most surprisingly, differences in fecundity and offspring quality were even manifested across years. CORT-clipped mothers from 2005 that returned the following year fledged significantly more young of higher quality than did sham-clipped mothers, despite both groups having laid similar clutch sizes and hatching similar numbers of young in 2006. These results support our main hypothesis that prenatal corticosterone exposure of offspring "rescued" clipped mothers from potential negative effects on fitness by better matching these mothers to their offspring. While studies manipulating current reproductive effort have shown that both intra- and interannual costs to fecundity do occur (Røskaft 1985; Lessells 1986; Gustafsson and Sutherland 1988; Nur 1988; Young 1996; Golet et al. 2004; Hanssen 2005), to our knowledge, these results are the first to report interannual effects of individual variation in maternal condition, especially as mediated by a maternally derived hormone.

In addition to effects on future fecundity, the trade-off between current reproductive effort and survival predicts that increasing reproductive output will negatively impact maternal survival (Stearns 1992), and numerous experimental studies have attempted to examine this life-history trade-off, with varying results (Nur 1984, 1988; Dijkstra et al. 1990; Daan et al. 1996; Young 1996; Golet et al. 1998, 2004). Given the better "match" between maternal quality and offspring demand for CORT-clipped versus sham-

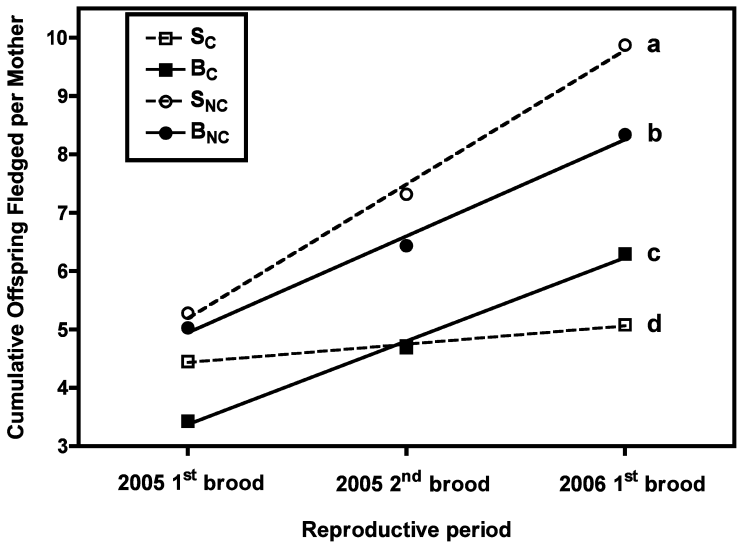

Figure 6: Average cumulative offspring fledged for European starling mothers from the original treatments over the three recorded reproductive attempts (different letters represent significant differences between groups; treatments: sham-clipped [S_C], CORT-clipped [B_C], sham-nonclipped [S_{NC}], and CORT-nonclipped [B_{NC}]).

clipped mothers, we predicted that the lower current reproductive investment for CORT-clipped mothers would increase their local survival. This prediction was confirmed, with CORT-clipped mothers manipulated in 2005 having a higher rate of local survival to 2006 and 2007 than sham-clipped mothers. While it is tempting to suggest that the loss of body condition following first and second broods in 2005 is the mechanism linking reduced investment in the current brood and increased local survival in these mothers, there are clearly many possible mechanisms. Some studies have proposed that it is the direct loss of condition that causes the decrease in survival (Golet et al. 1998), while other studies have separated condition changes from food quality/availability following the breeding season and have found that condition may not play a direct role (Golet et al. 2004). Other studies have experimentally increased current reproductive effort and have found that decreases in underlying physiological processes such as immune function (Ardia et al. 2003; Hanssen et al. 2004) and allostatic load (Golet et al. 2004) may actually mediate the relationship between condition and survival. We are currently examining baseline corticosterone levels and oxidative stress in relation to maternal workload in our study system (S. Bourgeon, O. P. Love, and T. D. Williams, unpublished data) to examine underlying physiological traits that may be driving these effects on survival.

Finally, to understand how maternal fitness was affected by our experimental manipulations in 2005, we combined fecundity (number of young produced/attempt and reproductive success) with maternal survival to calculate the average cumulative number of independent offspring produced by mothers assigned to each group. This analysis confirms that CORT-clipped mothers have higher fitness than do sham-clipped mothers through three successive postmanipulation breeding attempts. We consider this calculation to be quite conservative, since the quality of offspring raised by CORT-clipped mothers was significantly higher than that of offspring of sham-clipped mothers in all reproductive attempts, and it is well known that offspring quality affects survival (reviewed in Starck and Ricklefs 1998; McCarty 2001). As such, we would expect significant differences in juvenile fitness between the groups as well, resulting in even higher fitness for CORT-clipped versus sham-clipped mothers. Overall, our results indicate not only that low-quality mothers suffer long-term fitness costs (fecundity, offspring quality, and survival) if they raise a brood that is not optimally prepared and matched to their rearing capacity, but also that exposing their offspring to a hormonal signal that correlates with their quality (maternally derived yolk corticosterone) can modulate these costs, proximately providing a better "match" between mother and young and ultimately improving maternal fitness.

Evolutionary Drivers of Corticosterone-Induced Male Phenotypic Plasticity

Producing an optimal offspring sex ratio at laying may be difficult or even maladaptive, despite evidence that it is occurring in some species (Appleby et al. 1997; Sheldon

Table 3: Residual effects of the 2005 maternal treatments on phenotypic traits of starling fledglings in first broods of 2006

Traits (in 2006)	S_C	B_C	S_{NC}	B_{NC}
Body mass (g)	77.68 ± 2.35^A	78.54 ± 1.23^A	$78.10 \pm .91^A$	79.69 ± 1.16^A
Tarsus (mm)	$33.95 \pm .36^A$	$34.78 \pm .19^B$	$35.08 \pm .14^B$	$34.75 \pm .18^B$
Wing cord (mm)	76.37 ± 2.39^A	82.25 ± 4.56^B	84.63 ± 1.76^B	83.47 ± 2.26^B

Note: Different letters represent significant differences within sexes across treatments. Treatments: sham-clipped (S_C), CORT-clipped (B_C), sham-nonclipped (S_{NC}), and CORT-nonclipped (B_{NC}).

et al. 1999; Komdeur et al. 2002; but see Ewen et al. 2004), since the correlation between environmental cues at laying and conditions during chick rearing might be low and unpredictable (Nager et al. 2000). Alternatively, a flexible hormonally mediated mechanism of sex-specific, quality-mediated alterations in offspring phenotype may provide better fitness returns than would fixing the sex ratio at laying (Love et al. 2005). Our results indicate that a single factor, male sensitivity to yolk corticosterone during embryonic development, can have a cascading effect on maternal fitness measures spanning current and future breeding attempts. However, why do male offspring (but not females) exhibit phenotypic plasticity (specifically lower hatch masses) in response to elevated yolk corticosterone if this response increases their chances of postnatal mortality? At present, we can think of two possible hypotheses to explain this phenomenon. First, this might represent a "predictive adaptive response" (reviewed in Gluckman et al. 2005; but see Wells 2007), an idea based on the "thrifty phenotype" hypothesis developed by Hales and Barker (2001; Barker 2002) for mammals. The thrifty phenotype hypothesis is an immediate fetal adaptation proposed in mammals (e.g., altered energetic demand/reduced fetal growth) to altered nutrient supply from the mother designed to increase the survival of the embryo to parturition. Predictive adaptive responses are a form of phenotypic plasticity (Pigliucci 2001; West-Eberhard 2003) hypothesized to have evolved as adaptive responses to environmental cues acting early in the life cycle but where the advantage of the induced phenotype is primarily manifest in a later phase of the life cycle; the cue thus acts as a predictor of the nature of the future environment (Gluckman et al. 2005). In mammals, exposure to maternal glucocorticoids in utero has been proposed to act as a predictive adaptive response, with this hormonal cue providing offspring with information regarding the relative stressfulness of their mother's environment; offspring would thereby alter their phenotype to better match their mother's quality (Seckl 2001, 2004; Gluckman et al. 2005). Although starling sons and daughters usually hatch at similar body masses, sons grow to a larger body mass and structural size and do so by having higher postnatal developmental rates than their sisters (Love et al. 2005; Verspoor et al. 2007; data presented here). If sons can interpret yolk corticosterone as a cue of their mother's low quality, they may be able to modulate fetal growth, resulting in a lower hatching mass, reduced begging, and therefore lower energetic demands. However, this suggests that modulating embryonic growth increases the risk of mortality, since CORT-clipped sons experienced elevated mortality in this study. This might arise from an even further (unpredictable) drop in the quality of the postnatal developmental environment, meaning that sons may not be able to perfectly predict how poor their postnatal developmental environment will be. Importantly, if these phenotypic responses are adaptive for males, it is still perplexing why sons of sham-clipped mothers experienced lower mortality and were of comparable quality to males raised by CORT-clipped mothers. That is, why would any male modulate its hatching phenotype in response to elevated maternal corticosterone if it risks higher mortality and apparently gains nothing directly? Why wouldn't a son take the opposite approach and hatch as a "supermale" ready to outcompete his sisters? The answer likely requires (1) a complex understanding of the short-term costs and benefits of attempting to outcompete siblings, (2) an examination of the costs of a combination of the longer-term effects on male fitness of having to compete against both many brothers and many sisters, and (3) an understanding of the potential benefits that a son gains through inclusive fitness if his high-quality sisters can survive to reproduce.

A second, mechanistically simpler alternative explaining why male hatching phenotype is adjusted following exposure to elevated yolk corticosterone was recently proposed by Love et al. (2005) and is based on the idea that faster-growing sons (Verspoor et al. 2007) may have no choice but to respond to maternal corticosterone, since the hormone is involved in the prenatal programming of many developmental pathways. Elevated glucocorticoid levels can inhibit cell proliferation and growth (Orth et al. 1992) because they downregulate the release and activity of pituitary growth hormone and insulin-like growth factors, reduce the ability of embryos to regulate levels of glucocorticoid receptors (Tonshoff and Mehls 1997; Li et al. 1998; Woodall et al. 1999; Ghosh et al. 2000; Nolan et al. 2001; Seckl 2004), and interact with developmental hor-

mones such as thyroid hormones (de Jesus et al. 1990; Redding et al. 1991). Glucocorticoids also act as transcription factors since many genes have glucocorticoid response elements on DNA, and thus any potential change in glucocorticoid levels can have profound effects on development (for review, see Byrne 2001). In a sexually size-dimorphic species, these developmental effects could be sex specific, given that the sexes may require different levels of growth hormones, insulin-like growth factors, or even receptors for these hormones during prenatal or postnatal development. Since larger adult male size ultimately provides fitness benefits to not only sons but also their mothers, the need for an individual son to grow larger and faster during postnatal development could be co-opted by mothers taking evolutionary advantage of male sensitivity to prenatal glucocorticoid-dependent developmental pathways (Love et al. 2005). If this were the case, one may expect there to be parent-offspring conflict between males and mothers as sons attempt to break from their mother's developmental control (Crespi and Semeniuk 2004; Wendt Müller et al. 2007). However, if glucocorticoid-mediated developmental pathways are fixed and likely evolved before starlings became sexually size dimorphic, then males may not be able to developmentally ignore the presence of elevated yolk corticosterone. Complex molecular studies of differences in growth factors, growth hormones, and receptor density will begin to disentangle the two mechanistic hypotheses presented here and will help to address further technical differences between adaptation, exaptation, and constraint (Ketterson and Nolan 1999).

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